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Insects as vectors of yellow dwarf, a virus disease of onions

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INSECTS AS VECTORS OF YELLOW DWARF, A VIRUS DISEASE OF ONIONS

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INTRODUCTION

In the spring of 1927 onion growers in eastern Iowa became very much concerned over a malady which was causing serious losses to the onion crop. An investigation revealed the presence of an unknown disease. During the succeeding year (1928) the malady attained epiphytotic proportions, 30 to 40 per cent of the commercial plantings of the district being affected and in some fields more than 90 per cent of the plants became diseased. How and when it reached this territory is not definitely known. Information obtained from growers and others interested in onion production in this area lead to the conclusion, however, that the disease had probably been present for a period of several years but unnoticed because of its relatively mild proportions.

The symptoms of the disease and the lack of any constantly associated organism in combination with a demonstration of its transmissibility strongly indicated that the causal agent was a virus. The writer was employed by the Entomology Section of the Iowa Agricultural Experiment Station to study the entomological aspects of the problem, with reference to the part played by insects in the natural dissemination of the disease. These studies have been conducted over

a period of several years both under field conditions in the Pleasant Valley onion growing district and in the greenhouse and experimental fields at Ames.

REVIEW OF LITERATURE

Yellow dwarf is a comparatively new communicable disease of the cultivated onion and literature pertaining to it is rather meager.

It was first described and referred to as "yellow dwarf" by Melhus et. al. (1928) and evidence indicating it to be of the nature of a virus was presented. Evidence that onion dwarf may be transmitted by aphids was first presented by Drake, Harris, and Tate (1932). In succeeding publications (1933 and 1934) the same authors presented evidence to show that the disease may be transmitted by a large number of different species of aphids but they were unable to consistently secure transmission with any other group of insects. A bulletin dealing with the various phases of the disease from a phytopathological standpoint has recently been published by Henderson (1935).

GEOGRAPHICAL DISTRIBUTION

In the "Plant Disease Reporter" for 1918 a mosaic of onions is reported as having been observed in West Virginia during the previous year (1917). During the succeeding year (1918) the disease caused a moderate amount of damage in two counties. In the same publication for 1929 it is stated that the disease is now considered by N. J. Giddings as being identical with that occurring in Iowa.

According to Bremer (1929) there is in Germany a disease of onions which is characterized by a drooping, crinkling, and yellowing of the leaves associated with metabolic disturbances leading to a reduction of bulb and seed formation. The malady is referred to as "Rotzkrankheit" or slime disease. During 1929 the disease is said to have been prevalent in various parts of Germany and as high as 95 per cent infection was noted in some fields. This disease appears to be quite similar, if not identical, to that which occurs in Iowa.

Blattny (1930) reports that a disease of onions, referred to as onion yellows, occurs sporadically in Czecho-Slovakia. It is considered as being of virus origin and the characteristics of the disease appear to be somewhat similar to that of onion yellow dwarf in Iowa.

Henderson (1935) reports that specimens of yellow dwarf diseased plants have been collected both in Minnesota and in California.

DESCRIPTION OF THE DISEASE

In general the symptoms of yellow dwarf vary considerably with the time of infection, and growing conditions of individual plants.

The initial symptoms on plants grown from infected bulbs are apparent as chlorotic streaks or blotches only slightly lighter in color than the surrounding normal appearing green tissue and suggest a mosaic type of disease. As growth continues chlorosis becomes more pronounced and the spots elongated, thus producing a somewhat streaked appearance. Under some conditions and especially in more mature plants, a general chlorosis may be developed in the affected parts. In advanced cases the plants are severely stunted, the leaves crinkled, flat, and often drooped with the tips touching the ground.

Plants which become infected during the current season develop the first disease symptoms on the central or younger leaves 5 to 12 days after inoculation in the form of yellowish blotches or abbreviated streaks, which are irregular in outline, and give the affected leaves, in general appearance, a mild mosaic pattern. The degree of manifestation increases from a barely recognizable discoloration at first

to a more or less pronounced streaking and chlorosis may become systemic in the above ground portions of the plant. Occasionally, and especially under greenhouse conditions, plants that originally showed typical yellow dwarf symptoms may change to such an extent that the entire plant becomes a somewhat deeper green color than normal tissue and exhibit a decided glossy appearance. The degree of stunting and amount of injury to the current growth varies considerably with the stage of growth of the plant and is much greater when infection occurs early.

The initial symptoms on flower stems of infected plants are apparent as more or less elongated streaks or blotches extending from the base upward. As the disease progresses the streaks coalesce, thus producing a general chlorotic appearance. In advanced stages the flower stem becomes very much twisted and curled, and fails to produce a normal seed crop.

Plants which are fairly mature at the time of exposure suffer little damage during the current season and may or may not show any evidence of the disease but if such bulbs are planted the following season the result will be a very much reduced yield and the quality of an inferior grade.

Masking of symptoms is of common occurrence, especially under unfavorable conditions of growth and in plants which

are fairly mature at the time infection takes place. Since there are no visible indications of the disease in such cases, its presence can be detected only by regrowing the bulbs or by using the plants as a source of inoculum for infecting other plants. Further reference to this subject will be made in a subsequent section of this report.

In old plants which have become infected late in the season, and fail to develop marked yellow dwarf symptoms, it is quite often difficult for the inexperienced observer to determine whether a suspicious plant is diseased or has been severely attacked by the onion thrips. The injuries are somewhat similar in that the leaves are drooped often with their tips touching the ground. There is, however, a very distinct difference between the two maladies (Plate I). The feeding activities of individual thrips results in the production of minute lesions on the leaves which may later develop into necrotic spots. As the extent of the damage increases, these lesions become more numerous and often confluent, thus producing the well known condition commonly referred to as "white blast" or "silver top", a condition which has been described in detail by Harris, Drake, and Tate, (1936). The seriousness of the injury is later manifested by a curling and gradual drying of the leaves which, however, retain their rigidity to some extent. In the case of yellow dwarf symptoms, there is an absence of spotting and the characteristic whitish

color produced by thrips. Also, the leaves, instead of being rigid, are somewhat soft and flabby.

SUSCEPTS

So far as has been definitely established no wild host plants of the onion yellow dwarf virus have been found. Thus, the disease under normal conditions is limited entirely to the cultivated onion. There is some evidence of variation in susceptibility of different varieties of cultivated onions and one variety, the Riverside Sweet Spanish type, showed considerable resistance. The author, however, obtained from 30 to 80 per cent infection in a number of experiments with this variety when insects were used as the transmitting agent. Attempts to transmit yellow dwarf to wild onion and various other species of plants, both wild and cultivated, were unsuccessful. Henderson (1935) reported successful inoculation of the Chinese sacred lily (Narcissus tazetta L.), Jonquil (Narcissus jonquilla L.) and shallots (Allium ascolanicum L.) with yellow dwarf virus. He does not state, however, whether or not the virus could be transmitted from any of the infected plants back to the onion or from host to host among these plants, a condition which leaves the possibility of such plants serving as a host of the yellow dwarf virus in a rather dubious state.

PRELIMINARY STUDIES OF INSECT TRANSMISSION

Because of the similarity of yellow dwarf of onions to certain insect-borne diseases, such as sugar cane mosaic, aster yellows and others, it seemed logical to suspect that insects were responsible for its natural dissemination. A survey of the insect pests attacking onions in Iowa, including both primary and secondary forms, was made for the purpose of determining which ones might be involved in spreading the disease. For many years the onion thrips, Thrips tabaci Lind. has been a more or less serious onion pest in this state and, since it is known to serve as a vector of certain other virus diseases, such as pineapple yellow spot (1932), attention was immediately directed to this pest. The tarnished plant bug, Lygus pratensis L. was found to be fairly abundant during certain seasons as were also several species of leaf hoppers, including Cicadula sexnotata Fall, Deltocephalus inimicus Say and Empoasca fabae Harris.

In addition, the bulb mite, Rhizoglyphus hyacinthi Boisd, the imported onion maggot, Hylemyia antiqua Meig, the black onion fly, Tritoxa flexa Wied, the seed corn maggot, Hylemyia ciliorura Rond., the barred wing onion fly, Chaetopsis aenea Wied, the lesser bulb flies, Eumerus strigatus Fall, and

E. tuberculatus Fall and a number of other dipterous maggots of secondary importance were known to occur in considerable abundance during certain seasons.

In order to test the possibility of insect transmission a number of experiments were undertaken. In these tests either the Red Globe or Yellow Bottleneck varieties of onions, which are the most common commercial varieties grown in the Pleasant Valley district, were used.

Experiments With the Onion Thrips, Thrips tabaci. Lind.

In view of the fact that the onion thrips, T. tabaci, comprises the most abundant and widely distributed insect pest of onions in Iowa it was regarded with suspicion and it seemed advisable to thoroughly investigate the possibility of its serving as a vector of the yellow dwarf virus. For conducting the inoculation tests, which have extended more or less interruptedly over a period of four years, various types of cages and procedures have been employed.

In one series of tests an insect proof cage (4' x 4' x 4'), which was aerated by means of an electric fan, was used to confine the thrips. Nymphs of T. tabaci were transferred to diseased onion plants within the cage and these served as the initial stock supply. After these had increased to the

point of a fairly heavy infestation by breeding on diseased onion, healthy sets, from which all insects had been removed by sterilization methods, were planted inside the cage. These plants were left inside the infection cage for a period of about 7 days, after new growth had started, and then removed to a more favorable growing environment in an "insect free" room of the greenhouse and examined daily over a period of 40 to 50 days for the development of yellow dwarf symptoms. By this method it was possible to maintain in the cage a supply of thrips which had developed for several generations entirely from eggs deposited in diseased onion tissue and which had been compelled to feed during a greater part of their nymphal and adult existence on infected plants. These tests which included 212 plants resulted in no successful virus transmissions.

Similar experiments were conducted in which diseased onion plants growing in the cages were permitted to become infected with thrips. After a heavy population had developed, healthy sets were planted in the cages alongside the thrips infested diseased plants and there grown to maturity. By this method approximetely 500 healthy plants were exposed but no transmissions occurred.

The bulbs of 200 plants which were exposed to thrips in the above experiments and then grown to maturity were planted in the greenhouse the following season to determine

if disease symptoms might develop during the second growth period. All of these plants remained healthy.

During the winter months (Febr., March and April) of 1932 an experiment was conducted in which an "insect proof" compartment of the greenhouse was used as the experimental cage. Diseased bulbs were planted at weekly intervals in the soil benches and allowed to become heavily infested with onion thrips. Approximately 500 healthy bulbs were then planted among the diseased plants so that there would be an opportunity for the thrips to migrate in a normal manner from diseased to healthy plants. The healthy plants readily became infested but no yellow dwarf symptoms developed during the 3 months that they were kept under observation. Approximately 250 of these bulbs were regrown the following fall but no evidence of transmission was obtained.

Duplicate series of experiments, involving a total of more than 1000 plants, about half of which were carried through a second growth period, were later conducted but with negative results.

A number of other types of cages which it is not thought necessary to describe at this time were employed. A total of more than 3000 plants were exposed to nymphs and adults of T. tabaci which had fed for 2 or more successive generations on diseased onion but no successful transmissions were obtained.

These negative results together with confirmatory field evidence indicate that this insect is unable to transmit the virus of onion yellow dwarf from diseased to healthy plants.

Experiments With Leafhoppers

Although there are no species of leafhoppers which are known normally to breed on the onion plant or even feed on it to any great extent in Iowa, field observations show that certain species such as C. sexnotata, D. inimicus, E. fabae and others often may be found in onion fields in considerable numbers.

In June 1931 a series of experiments were conducted to determine if C. sexnotata, which could be collected in large numbers by sweeping with an insect net in onion fields, were capable of serving as vectors of the onion yellow dwarf virus. Approximately 1/4 of an acre of land located about 2 miles from the infected area was obtained for use in transmission studies. Set onions produced in disease free areas were planted at frequent intervals to insure a constant supply of susceptible host plants in the most desirable stage of growth. Large numbers of leaf hoppers were collected 3 to 5 times each week for several weeks, either in onion fields or nearby in the infected area, and confined on diseased onion plants for varying intervals of time (1, 2, 3, and 4 days).

In some experiments the leafhoppers were removed from the diseased plants and caged directly on healthy plants in the experimental plots, whereas, in others, they were caged on their normal host plants, after being confined on diseased plants, for varying periods of time up to 11 days and then transferred to healthy onions. The cages usually remained over the experimental plants until all insects had perished, which was ordinarily 4 or 5 days, but in case any were still alive at this time they were destroyed either by hand or by means of sprays in order to prevent chance inoculation. A total of 340 plants were exposed to C. sexnotata in this manner but none were visibly infected at the time of harvesting the bulbs during the early part of July (1931).

Parallel experiments involving 205 plants were conducted with D. inimicus but, likewise, the results were negative.

All of the plants exposed to the 2 species of leafhoppers mentioned above were brought to Ames and regrown during the following December (1931) in the greenhouse in order to determine if yellow dwarf symptoms might develop during the second growth period. All results were negative.

Somewhat similar experiments were conducted with C. sexnotata during July, August, and September 1931 in the experimental plots at Ames.

Altogether 212 healthy onion plants were exposed to

leafhoppers which had previously fed on diseased onion plants with negative results. These plants were also harvested and regrown in the greenhouse during the following winter months but none of them developed yellow dwarf symptoms.

During the fall and winter of 1931-32 the leafhopper C. sexnotata was cultured on asters in "insect proof" cages in the greenhouse at Ames. The original stock supply was obtained by collecting approximately one dozen adults from blue grass and confining them on young aster plants in cages. Within a period of a few weeks a practically unlimited supply was available. Colonies were maintained in several different cages at all times. Some difficulty was experienced because of chance infestations of the aster plants by the greenhouse white fly, Trialeurodes vaporariorum Westwood, and red spiders, Tetranychus telarius Linn. Whenever such infestations occurred, the plants and insects were destroyed and the cage thoroughly fumigated.

In conducting the transmission experiments the leafhoppers were confined on diseased plants in large lantern globle cages for varying periods of time, depending upon the individual experiment in progress. In some cases they were transferred directly from the diseased onion plants to healthy onion plants while in others they were confined on aster plants for a given interval of time before being transferred

to healthy onion plants. In some instances the insects were transferred from diseased plants to a succession of healthy onion plants. The idea involved in the various methods of handling the insects was to take advantage of the fact that there might be a period of delayed infectivity or so-called incubation period in the leafhoppers such as has been shown to occur, by Kunkel (1926), in connection with aster yellows and the aster leafhopper, C. sexnotata.

Through the procedures herein described 512 healthy onion plants were exposed but, with one exception, all remained healthy during the period which they were kept under observation. The one plant which became diseased in this series seems to be the result of an accidental transmission since numerous attempts to obtain additional transmissions have been unsuccessful.

From these experiments it is concluded that neither C. sexnotata nor D. inimicus are capable of transmitting the virus of onion yellow dwarf from diseased to healthy plants.

It should be noted that, although the two above mentioned species of leafhoppers do not normally select the onion plant as a host, they feed quite readily when confined on this plant and many of them lived for a week or more under such conditions.

Experiments With the Potato Leafhopper, Empoasca fabae Harris

As mentioned in a previous section of this report, the potato leafhopper often is found in onion fields in considerable numbers, especially when its host plants are growing in adjoining or nearby areas.

During June and July 1931 potato leafhoppers, which could be collected in large numbers on potato plants, were confined on diseased onions for an interval of one or two days and then transferred to healthy onion plants. As a rule the cages remained over the experimental plants until all insects had died, which was usually from 3 to 5 days. In some cases the transfers were made from diseased onion back to potato plants for an interval of time before exposure of healthy onion plants. Of 147 healthy plants exposed none developed yellow dwarf symptoms during the current season or when they were regrown in the greenhouse.

Experiments With the Tarnished Plant Bug, Lygus pratensis L.

The tarnished plant bug, which may be classed as a somewhat omnivorous feeder, often occurs in onion fields in large numbers.

On onion plants the injury caused by the feeding of the

tarnished plant bug first becomes apparent as a small "water-soaked" area which gradually turns brown and may develop into a necrotic spot. In the onion growing district at Pleasant Valley such injury has been observed to become so widespread as to result in somewhat serious damage in local areas. They quite commonly congregate in large number in the flowers of onion plants and their feeding activities may cause a serious reduction in yield.

Although the tarnished plant bug often has been suspected as a vector in connection with a number of plant viruses there are no cases to date of its having been positively incriminated with the exception of a few easily transmissible diseases (e.g. potato spindle tuber, Goss, 1928). Its abundance in onion fields in combination with its suctorial feeding method, however, quite naturally placed it under suspicion as a vector of yellow dwarf.

During the spring and fall of 1931 and 1932 a series of experiments were conducted in which tarnished plant bugs were confined on diseased onion plants for 24 hour periods, as a rule, and then transferred to healthy plants. In these experiments it was found desirable to use large cages (1' x 1' x 2') covered with cheese cloth which served as a partial shade. When confined in small glass or lantern globe cages, without complete shading, the bugs died within a very short time.

If more than 6 or 8 individuals were confined on a small onion plant for a few hours their feeding activities frequently produced such severe effects as to either kill the plant completely or cause a marked stunting. This being the case, the experimental plants were usually caged with 3 or 4 bugs which were removed after a 12 or 24 hour period.

A total of 94 healthy plants were caged with the tarnished plant bug with negative results.

Experiments With Onion Mirids, Labopidia ainslei Knight and L. alli Knight

Two species of plant bugs, L. ainslei and L. alli, which feed and breed on onions, both wild and cultivated, and a few closely related plants such as garlic, occur quite commonly in Iowa. Both species were originally described by Dr. H. H. Knight in 1923 and 1928 respectively. Because of their small size (about 1/5 size of tarnished plant bug) and greenish color they are quite inconspicious when on their normal host and there appears to be very little damage resulting from their feeding activities in most seasons. However, in 1936 serious damage was done in some fields in Iowa.

Although a detailed biological study of these 2 species of insects has not been made, it has been determined that only one generation is produced each year. The active stages,

including nymphs and adults, are present for only a short time in early spring, being present the remainder of the year as eggs which are deposited in the host plant tissues. So far efforts to culture these insects in the greenhouse have not been successful.

Because of the peculiar life cycle here involved, transmission experiments were necessarily confined to the spring months. During the spring of 1930, 1931, 1932, and 1933 approximately 468 nymphs and adults, collected in the field, were caged on diseased onion plants for a period of 2 days and then transferred to large "insect proof" cages containing young healthy onion plants. All of these which included 74 plants, remained healthy during the current growth period and when regrown the following season.

Experiments With the Bulb Mite, Rhizoglyphus hyacinthi Boisd.

Under favorable conditions of temperature and humidity the bulb mite, R. hyacinthi, constitutes a pest of serious consequence to the onion crop, especially to stored bulbs and bulbs which are allowed to remain in the field sometime after becoming mature. Since it is a pest so intimately associated with the onion crop and one that appears to be continuously present, the possibility of some relationship

between it and the spread of yellow dwarf seemed tenable.

During the spring, summer, and fall months of 1930 and 1931 a series of transmission experiments with the bulb mite were undertaken. Inoculated bulb mites, obtained both on diseased plants which had become infested under natural conditions and on plants which had been experimentally infested, were transferred along with the diseased bulbs to healthy plants in "insect proof" cages. The plants remained caged for a period of 2 to 3 weeks. Of approximately 200 healthy plants exposed in this manner none developed yellow dwarf. About half of these plants were kept under observation during a second growth period but all remained healthy.

Experiments With Bulb Infesting Maggots

The imported onion maggot, H. antiqua, and the seed corn maggot, H. cilicrura, although not pests which occur in serious proportions each year in Iowa, may occasionally attain such numbers as to destroy 5 to 15 per cent or more of the onion crop. In general, such a condition prevailed during the years (1928-31) when the yellow dwarf disease was widespread in the Pleasant Valley onion growing district.

In spite of the fact that insects of this type appear to be poorly adapted for transmitting a virus disease and are not known to have been previously implicated in any instance,

the importance and close relationship of these two species of dipterous maggots to the onion crop suggested a possible relationship.

For confining the maggots on healthy plants, and, at the same time to prevent the entrance of other insects, large glass cages (1' x 2 1/2'x 4') were constructed and aerated by means of a small electric fan. These cages were placed over a group of plants, which were growing in a greenhouse bench, and inoculated maggots, obtained from either naturally or experimentally infested diseased bulbs, introduced. After a period of about 2 weeks the cages were removed and the plants kept under observation until time of harvesting. As was expected a few of the experimental plants were killed as a result of the maggot attacks and others were severely stunted. More than 200 plants were exposed in this manner with negative results. A considerable number of these plants were regrown but no yellow dwarf symptoms developed.

During the spring of 1931 a series of experiments were conducted in which inoculated maggots, obtained from naturally infested diseased bulbs, were introduced into small incisions made in the bulb of young healthy growing onion plants. Of approximately 250 plants exposed by this method none became diseased.

Since the imported onion maggot and the seed corn maggot often occur as mixed infestations and are rather difficult to

separate in their larval stages, no effort was made to isolate the two species in the above experiments.

Similar experiments were conducted at the same time with the black onion fly, T. flexa, and the barred-winged onion fly, C. aenea, but, since all results were negative, the details of the experiment will not be given.

Experiments With the Greenhouse White Fly, Trialeurodes vaporariorum Westwood

During transmission experiments with the various insects under greenhouse conditions it was noted that certain plants, such as tomato, aster, and others, often became heavily infested with the greenhouse white fly. In some instances large numbers of the adults of these insects were observed on onion plants growing nearby and many of them appeared to be feeding.

In April 1933, three series of experiments were conducted in which several hundred adult white flies were confined on a diseased onion plant for a period of 24 hours and then transferred to healthy plants in small lantern globe cages. Of 22 healthy plants exposed in this manner none became diseased.

Similar experiments were conducted with the red mite, T. telarius, with negative results.

Experiments With Mealy Bugs

While conducting transmission experiments with the various groups of sucking insects, it was thought advisable to include among this list certain species of mealy bugs which commonly occur on greenhouse plants and are practically always available in large numbers.

In March 1933 mealy bugs (*Phenacoccus* sp.) were collected from plants growing in the greenhouse at Ames and confined on diseased onion plants, an effort being made to select the younger and more active stages. After 24 hours they were transferred to healthy onion plants. While confined on the plants it was noted that many individuals did not appear to be feeding and considerable numbers were congregated either on the side of the cage or on the ground surface.

Twenty-eight healthy plants were exposed in this series of experiments and all remained healthy, with one exception. Additional experiments were later conducted in an effort to verify the previous transmission but without success. It is concluded, therefore, that the one diseased plant either was the result of an accidental infection from some other source or that transmission by mealy bugs is an extremely rare occurrence.

Experiments similar to the above with a mealy bug,

Pseudococcus citri Risso, collected from coleus plants, also gave negative results.

Experiments With Aphids

Previous to the outbreak of the yellow dwarf disease in Iowa there was little occasion for giving serious consideration to insects which only incidently occur on onion. With the advent of yellow dwarf, however, and the failure to incriminate any of the primary and secondary onion pests as vectors of the virus it was somewhat imperative that attention be directed toward insects which only occasionally or incidentally feed upon the virus host. Since aphids comprise perhaps the most important group of plant virus vectors, at least from the standpoint of number of diseases transmitted, attention was quite naturally directed toward this group of insects as possible vectors.

During the period from 1928-31 field observations showed that many different species of plant lice were present in onion fields either breeding on weed hosts or as migrants and that these insects fed on growing onion plants to some extent. A detailed discussion of this subject will be given in a subsequent section of this paper.

During December 1931 several hundred individuals of the bean aphid, Aphis rumicis L. which were being cultured on

nasturtium in a section of the Entomology greenhouse at Ames, were confined on a diseased onion plant for a period of 2 days and then transferred to young healthy onion plants. In this experiment 8 plants were used and at the end of approximately 3 weeks 5 plants had developed typical yellow dwarf symptoms. Previous to this time a few transmissions had been obtained with aphids but due to improperly controlled conditions the evidence was not convincing. Additional experiments were subsequently conducted with the same species and with a number of other species. A detailed account of these tests will be presented in the following discussion.

TRANSMISSION STUDIES WITH APHIDS

Experimental Methods

A stock supply of plant lice was maintained by colonization on one or more of their normal host plants grown either in small "insect proof" cages or in isolated compartments of the greenhouse. Transfers were made either with a small camel-hair brush or an aspirator similar to the type described by Kunkel (1926) for use in handling leaf hoppers. For confining aphids on diseased plants large lantern globe cages (see fig. 1, a) were employed. During the early part of the work cylindical glass tubes approximately 1-1/2 inch in diameter and 12 inches long and covered over with cheese cloth held in position by means of a rubber band were used to confine the insects on individual experimental plants (fig. 1, b, c, and d). Since there is a tendency for moisture to condense on the sides of small glass cages, when exposed to sunlight, celluloid and cellophane cages of a similar size and shape were substituted and these proved to be somewhat more satisfactory.

The aphids were confined on a diseased plant for a given period of time and then transferred directly to the experimental plant, as a general rule at the rate of 30 to 50

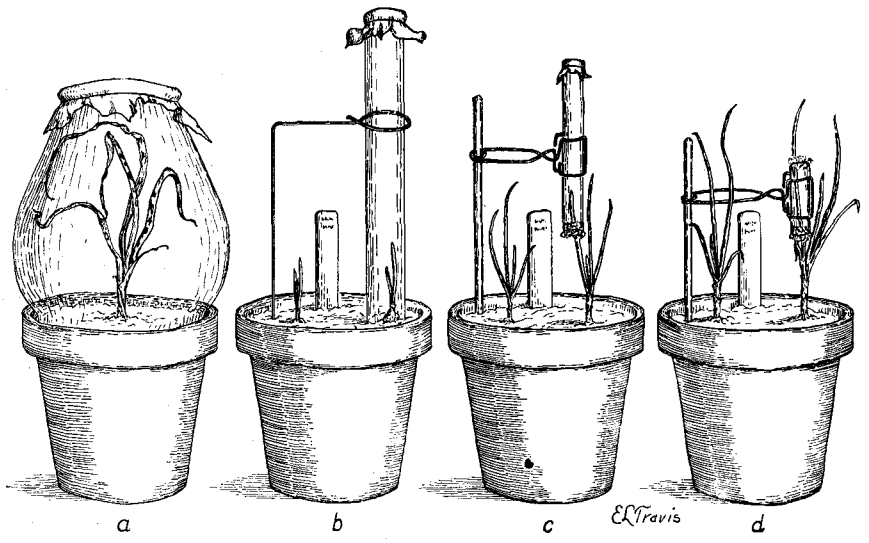

Fig. 1. Types of cages used for confining aphids on growing onions: a, aphids feeding upon diseased plant; b, c and d, cages adapted for confining viruliferous aphids upon entire or part of plants (control plants not caged).

individuals per plant. Except for certain types of experiments, which will be mentioned later in the text, the insects were kept on the experimental plant for a period of 2 or 3 days after which the cages were removed and the plants carefully sprayed with either a solution of pyrethrum or nicotine sulphate and soap. All experimental plants were accompanied by an equal or greater number of control plants.

Experimental plants were grown from sets, 3/8 to 1/2 inch in size, which were produced in disease free areas and no cases of natural infection were known to have occurred. As a rule the plants were used when one to four inches in height, more satisfactory results being obtained with plants of this age than with older or more mature plants.

To avoid injury to onion plants by thrips and to prevent chance infestations of weeds, which occasionally obtained a start in the experimental greenhouse, by other insects, it was found necessary to apply sprays at regular intervals. The use of either a nicotine sulphate-soap or pyrethrum spray proved satisfactory for preventing infestation by these pests. Occasionally the sprays were supplemented with fumigation by means of vaporizing free nicotine as an additional precaution against infestations of extraneous insects.

Transmission by Aphis rumicis

Because of the abundance, wide distribution and convenience with which it may be cultured, A. rumicis was selected as the most satisfactory species of aphid to use in large scale transmission studies. Vigorous colonies were maintained on dwarf nasturtium grown in flats(1' x 2' x 6") placed either in insect proof cages or in isolated compartments of the greenhouse. In order to have as nearly a homogenous population as possible, the stock supply of aphids was developed originally from a single parthenogenetic female.

The results of transmissions tests with A. rumicis during the seasons of 1931-32 appear in Table I. It will be noted that of 332 plants exposed 236 or an average of 71.0 per cent became diseased. The time required for the first symptoms of the disease to develop was relatively short, being 7 to 12 days in most cases with an average of 10.6, but the range was from 4 to 29 days. The incubation period of the disease is very markedly influenced by the age and state of growth of the plant and by the greenhouse temperature, and is comparatively longer in older plants in a poor state of growth and when the temperature is sufficiently high to retard the growth of the plants.

TABLE I. TRANSMISSION EXPERIMENTS WITH A. RUMICIS

Date exposed.	No. plts. exposed	No. Dis. plts. Checks	Exp. plts.	% Trans.	Av. Inc. Period
Dec. 9, 1931	1	0	1	100.0	29
Dec.10, "	3		2	66.6	28
Dec.21, "	1		1	100.0	18
Jan.14, 1932	11		3	27.2	14.3
Jan.15, "	7		3	42.8	12.3
Jan.17, "	1		1	100.0	12.0
Jan.18, "	6		3	50.0	11.6
Jan.19, "	9		2	22.2	11.0
Jan.22, "	1		1	100.0	10.0
Jan.23, "	5		1	20.0	11.0
Jan.26, "	3		1	33.3	8.0
Jan.27, "	5		1	20.0	8.2
Jan.28, "	5		3	60.0	10.0
Jan.30, "	8		6	75.0	10.8
Jan.31, "	5		3	60.0	8.3
Feb. 1, "	10		7	70.0	8.1
Feb. 3, "	8		5	62.5	7.0
Feb. 4, "	8		3	37.5	9.8
Feb. 7, "	6		5	83.3	9.4
Feb. 8, "	7		7	100.0	8.5
Feb. 9, "	6		3	50.0	9.0
Feb.10, "	8		7	87.5	8.0
Feb.11, "	11		10	90.9	8.4
Feb.12, "	5		4	80.0	11.0
Feb.13, "	8		6	75.0	8.1
Feb.14, "	3		3	100.0	8.0
Feb.15, "	9		8	88.8	10.1
Feb.16, "	9		9	100.0	11.0
Feb.17, "	10		5	50.0	13.4
Feb.18, "	7		3	42.8	13.6
Feb.19, "	8		6	75.0	9.1
Feb.20, "	13		10	76.9	8.1
Feb.21, "	11		9	81.8	7.5
Feb.27, "	2		2	100.0	10.5
Mar. 1, "	14		11	78.5	7.7
Mar. 2, "	9		5	55.5	13.0
Mar.21, "	12		7	58.3	8.3
Mar.27, "	6		3	50.0	7.6
Apr. 4, "	5		5	100.0	6.4
Apr. 5, "	6		6	100.0	6.1
Apr. 7, "	5		2	40.0	7.5
Apr. 8, "	12		11	91.6	6.4

Table I (continued)

Date exposed	No. plts. exposed	No. Dis. plts. Checks Exp. plts.	% Trans.	Av. Inc. Period
Apr. 9, 1932	10	10	100.0	6.2
Apr. 10, "	3	3	100.0	6.6
Apr. 11, "	10	10	100.0	10.2
Apr. 12, "	10	7	70.0	22.0
Apr. 27, "	2	2	100.0	7.2
May 5, "	4	2	50.0	14.5
May 6, "	2	1	50.0	13.0
Oct. 8, "	7	7	100.0	6.0
Total	332	236	Av. 71.0	Av. 10.6

Rapidity With Which A. rumicis Becomes Infective

A series of experiments were conducted to determine the period of time necessary for A. rumicis to feed on a diseased plant in order to become infective. With insects which do not normally feed on the virus host plant and apparently accept such plants as a source of food only during their wanderings or migrations, or in case of necessity due to the absence of their normal host plant, this point has considerable influence on the effectiveness with which the vector may disseminate the virus. In order to establish this relationship, colonies of A. rumicis were confined on diseased onion for varying periods of time and then transferred to healthy onion plants. In preliminary experiments it was determined that the period of time was relatively short, less than 48 hours. With this range established aphids were confined on diseased plants for periods of time varying from 30 minutes to 48 hours. The results of these tests are presented in Table II. It will be noted that there does not appear to be any significant difference in the per cent of transmission obtained within this range.

In one instance successful transmission was obtained by a single aphid which had been confined on a diseased plant for only 15 minutes. These tests established the fact that extremely short feeding periods on a diseased plant are sufficient for the aphid vector to become infective.

Table II RAPIDITY WITH WHICH A. RUMICIS BECOMES INFECTIVE WHEN FEEDING ON DISEASED PLANTS

Period of time on Dis. plts. in hrs.	No. healthy plts. exposed	No. plts. infected	% Trans.
48	64	48	75.0
24	53	40	75.4
12	32	21	65.6
5	16	9	56.2
1/2	34	22	64.7

Incubation Period of the Virus in A. rumicis

Experiments were undertaken to determine whether or not transmission of the disease involves an incubation period of the virus in the insect. In the case of incidental or transitory vectors, which is quite apparently the relationship between plant lice and yellow dwarf of onion, the question of whether or not there exists a delayed infective power appears to have an important bearing on the vectors ability to spread the disease under field conditions. Preliminary experiments indicated that, if an incubation period existed, it was less than 60 hours. Colonies of aphids were confined on diseased plants for periods of 3 to 12 hours and then transferred to a succession of healthy plants. On each healthy plant was confined approximately 50 aphids consisting principally of adult apterous forms. The use of large numbers of aphids is desirable since all the insects placed on diseased plants do not appear to become infective and when confined on healthy plants all individuals do not feed, especially during short exposures. Moreover, during the process of transferring aphids many of them become disturbed to such an extent that considerable time may be spent in crawling or wandering about within the cage in an effort to escape. The result of these tests appear in Table III.

In one experiment 6 colonies of aphids were confined on

diseased plants for 6 hours and then transferred to a succession of healthy plants at irregular intervals, including 3 transfers of 6 plants each. The first transfers were made directly from diseased plants to 6 healthy plants. After a period of 16 hours they were transferred to 6 other plants for a period of 24 hours after which the 3rd transfer of 24 hours was made. At the end of this period the experiment was discontinued. One plant in the first transfer became diseased while all others remained healthy.

In a second experiment similar to the preceding one, 6 colonies of aphids were confined on diseased plants for 5 hours. Four transfers were then made at irregular intervals of 1/2, 2-1/2, 18, and 45 hours, respectively, after which the experiment was discontinued. By reference to Table III, it will be seen that successful transmission was obtained with plants included in the first 3 transfers but none thereafter.

In a third experiment involving 6 colonies of aphids which were confined on diseased plants for 6 hours, 5 transfers were made at intervals of 1/2, 16, 5, 3, and 6 hours, respectively. Successful transmissions were obtained in all except plants of the last transfer.

In a fourth experiment including 6 colonies of aphids which were confined on diseased plant for a period of 12 hours, 3 transfers were made at 24 hour intervals. By referring to

Table III, it will be seen that successful transmission was obtained only with plants exposed during the first 24 hour interval.

In a fifth experiment, similar to the preceding ones, 5 transfers were made at 1/2, 2, and the last 3 transfers at 24 hour intervals. Four plants out of 6 used in the first transfer became diseased while all others remained healthy.

By reference to Table III, it will be noted that successful transmissions were obtained only during the first few hours after the insects had been removed from the diseased plant. In no case did transmission occur after the vectors had been removed from the diseased plant for a period greater than 21-1/2 hours and in most cases the successful transfers were obtained during the first 2 or 3 hours.

These data indicate that there is no so-called incubation period in the insect or at most an extremely short one, and that infective power is rapidly lost when aphids are confined on healthy susceptible hosts.

TABLE III INCUBATION PERIOD OF THE VIRUS IN A. RUMICIS

Colony	Hrs. on Dis. Plt.	Hrs. removed from Dis. plt. before healthy plt. exposed	Hrs. Healthy plt. exposed	No. plts exposed	No. infected.
A	6	0	16	6	1
"	"	16	24	6	0
"	"	40	24	6	0
B	5	0	1/2	6	1
"	"	1/2	2-1/2	6	4
"	"	2-1/2	18	6	1
"	"	21	45	6	0
C	6	0	1/2	6	3
"	2	1/2	16	6	5
"	"	16-1/2	5	6	1
"	"	21-1/2	3	6	1
"	"	24-1/2	16	6	0
D	12	0	24	6	2
"	"	24	24	6	0
"	"	48	24	6	0
E	3	0	1/2	6	4
"	"	1/2	2	6	0
"	"	2-1/2	24	6	0
"	"	26-1/2	24	6	0
"	"	50-1/2	24	6	0

Retention of Infective Power by Aphids

The foregoing experiment suggested another to determine the period of time over which the infective power of aphids is retained. From Table III it is quite evident that in the process of transfering viruliferous A. rumicis to a succession of healthy susceptible plants the infective power is rapidly diminished, and completely lost after a few hours. Two other procedures were followed in an effort to establish a further understanding of this relationship. By the first method aphids were confined on diseased plants and then transferred for a period of time to immune plants, after which they were transferred back to healthy susceptible plants. By the second method aphids were caged on diseased plants and then confined without food in constant temperature chambers for varying periods of time.

"Retention of virus when confined on immune plants".- Three species of aphids were used in this experiment-namely, the bean aphid,(A. rumicis) the cabbage aphid, (Brevicornye brassicae Linn.), and the corn leaf aphid, (Aphis maidis Fitch), the normal immune hosts employed being, nasturtium, young cabbage plants and corn plants, respectively. The results of these tests appear in Table IV. After being confined on diseased onion plants, colonies of A. rumicis were transferred to nasturtium for periods of time ranging from 2 hours to

139 hours and then transferred to susceptible plants (onion). By reference to table IV, it will be noted that in no case were successful transmissions obtained after the aphids had been confined on immune plants for more than 36 hours.

In the case of cabbage aphids B. brassicae, 24 of 48 healthy plants to which direct transfers were made developed mosaic while of 16 plants exposed to aphids which had fed on normal immune plants for an interval of 24 hours none became diseased.

Somewhat similar results were obtained with the corn leaf aphid. Of 16 healthy onion plants which were exposed immediately after removal of insects from diseased plant 10 became diseased while of 18 plants exposed to aphids which had fed on corn plants for an interval of 24 hours or more none developed mosaic.

TABLE IV RETENTION OF INFECTIVE POWER BY APHIDS

Hrs. caged on infected plant.	Hrs. on immune plant.*	No. onion plts. exposed.	Number infected	% Trans.
		Aphis rumicis		
24	2	12	2	16.0
"	12	6	0	0.0
"	17	18	2	11.1
"	24	24	0	0.0
"	36	6	1	16.6
"	43	12	0	0.0
"	48	12	0	0.0
"	67	12	0	0.0
"	72	6	0	0.0
"	91	12	0	0.0
"	119	12	0	0.0
"	139	6	0	0.0
		Brevicornye brassicae		
"	0	48	24	50
"	24	16	0	0.0
		Aphis maidis		
"	0	16	10	62.5
"	24	6	0	0.0
"	48	6	0	0.0
"	72	6	0	0.0

* Immune to yellow dwarf of onion.

"Retention of infective power by A. rumicis when confined without food".- The question of how long viruliferous aphids retain their infective power when confined without food has a rather important bearing on their capacity to disseminate yellow dwarf virus under field conditions. Under the influence of the migratory impulse or any other condition that may serve to initiate movement, plant lice in some instances undoubtedly subsist for considerable periods during which they take little or no food. In determining how long A. rumicis retains its infective power under such conditions colonies of aphids were confined on diseased plants for 6 hour periods and then transferred to a constant temperature chamber (70° F. and 75 per cent relative humidity) for varying periods of time ranging from 3 to 63 hours. The results of these tests appear in Table V. It will be noted that no successful transmissions were obtained after the aphids had been confined for more than 8 hours without food. Although a considerable number of individuals died within the first 24 to 48 hours many of them remained active for more than 2-1/2 days.

From this and the 2 preceding experiments it is apparent that plant lice retain their infective power for only a few hours after removal from diseased plant regardless of whether they are feeding on healthy susceptible hosts, immune hosts, or confined without food.

TABLE V RETENTION OF INFECTIVE POWER BY *A. RUMICIS* WHEN CONFINED WITHOUT FOOD

Hrs. without food	No. healthy plants exposed	No. plants infected
3	5	1
4	5	3
5	11	2
6	5	0
7	5	0
8	11	2
9	10	0
10	5	0
12	24	0
14	12	0
15	20	0
17	5	0
18	12	0
19	10	0
21	5	0
23	30	0
24	8	0
27	5	0
34	7	0
35	5	0
36	5	0
37	3	0
39	5	0
41	9	0
42	5	0
43	6	0
48	5	0
63	11	0

Transmission Experiments With Various Species of Aphids.

In the course of both field work and experimental work on insects as related to the transmission of yellow dwarf of onion many different species of aphids have been taken on the foliage of onion, weeds in onion fields, and cultivated and wild plants in bordering and nearby areas. In preliminary experiments it was found that several species of plant lice, in addition to the one originally involved,-namely, the bean aphid, A. rumicis, were capable of transmitting the virus of yellow dwarf from diseased to healthy plants. In view of this fact it became of considerable interest to know if any species of aphids that could be induced to feed on onion plants could transmit the virus. As a result various species of aphids were collected in the vicinity of Ames and Pleasant Valley, Iowa, confined on diseased onion for a 24 hour period, as a general rule, and then transferred to young healthy onion plants. The results of these tests appear in Table VI. Credit is due Mr. Floyd Andre of the Iowa Agriculture Experiment Station, for the greater part of the field and laboratory work involved in conducting and interpreting the results of this series. Credit is also due Dr. F. C. Hottes for the identification of a considerable number of the species of aphids and for verification of the authors determination of a number of others.

TABLE VI TRANSMISSION EXPERIMENTS WITH VARIOUS SPECIES OF APHIDS

Species of Aphid	No. Healthy plants exposed.	No. Dis. plts. Checks	Exper. Plts.	% Trans.
Amphorophora rossi H. & F.	12	0	5	41.7
Aphis ageratoidis Oestlund	8		5	62.5
A. cardui Linn.	4		2	50.0
A. decepta H. & F.	10		8	80.0
A. forbesi Weed	9		5	55.5
A. gossypii Glover	29		24	82.7
A. helianthi Monell	18		14	77.7
A. laburni Kalt.	12		9	75.0
A. maidis Fitch	94		73	77.6
A. oenotherae Oestlund	6		2	33.3
A. oestlundi Gillette	14		10	71.4
A. pomi DeGeer	26		23	88.4
A. rubi Kaltenbach	5		2	40.0
A. rumicis Linn.	332		236	71.0
A. sambucifoliae Fitch	12		3	25.0
A. viburnicola Gillette	7		4	57.1
Brevicornye brassicae (Linn.)	39		20	51.2
Calaphis betulella Walsh	13		8	61.5
Capitophorus flaveolus (Walker)	15		13	86.6
C. ribis (Linn.)	5		4	80.0
Chaitophorus quercicola (Monell)	14		7	50.0
C. viminalis Monell	8		4	50.0
Cinara pini Linn.	12		0	0.0
Drepanaphis acerifoliae (Thomas)	20		11	55.5
Eulachnus rileyi Williams	9		0	0.0
Hyalopterus atriplicis (Linn.)	48		30	62.5
H. pruni (Geoffrey)	14		9	64.2
Hysteroneura setariae (Thomas)	14		10	71.4
Macrosiphum ambrosiae (Thomas)	26		24	92.3
M. gei (Koch)	35		16	45.7
M. gravicornis Patch	17		15	88.2
M. impatiensicolens Patch	10		9	90.0
M. pisi (Kalt.)	80		65	81.2
M. purpurascens (Oestlund)	5		2	40.0
M. rosae (Linn.)	16		16	100.0
M. rudbeckiae (Fitch)	20		16	80.0
Microsiphum artemisiae (Gillette)	7		5	71.3
Monellia caryae (Monell)	10		8	80.0
M. carycella (Fitch)	10		7	70.0

TABLE VI (Continued)

Species of Aphid	No. Healthy plants exposed.	No. Dis. plts. Checks	Exper. Plts.	% Trans.
Myzocallis alhambri Davidson	10		6	60.0
M. asclepiadis (Monell)	16		12	73.0
M. ononidis (Kaltenbach)	14		9	64.2
M. cerasi (Fabricius)	7		2	28.5
Myzus persicae (Sulzer)	48		30	62.5
Periphyllus negundinis (Thomas)	4		1	25.0
Prociphillus fraxinifolii (Riley)	6		0	0.0
Rhopalosiphum nymphaeae (Linn.)	23		19	82.6
R. prunifoliae (Fitch)	121		87	71.9
R. pseudobrassicae (Davis)	10		8	80.0
R. rhois Monell	16		10	62.5
Thripsaphis balli (Gillette)	10		9	90.0

It will be noted that of 51 species tested 48 are recorded as having transmitted the disease, a number which is sufficiently large to conclude that practically any species of aphid is potentially a vector of onion yellow dwarf. In addition to those recorded in Table VI, transmissions have been obtained with a number of other species but it is not considered necessary to prolong the list at this time.

As would be expected all species of aphids, and especially those which normally feed principally on trees and shrubby plants, do not readily feed on onion plants. When large numbers were confined on onion plants, however, no difficulty was experienced in most cases in securing a reasonably high per cent of successful transmissions. Contrary to what might be suspected a number of the non-migratory forms such as M. alhambri, which normally feed throughout the year on oak trees, and M. asclepiadis, which is normally confined to milkweed species (Asclepias sp.) were collected in onion fields in large numbers during certain periods as alate forms.

Masked Symptoms; Visibly Uninfected Plants as a Source of Infection.

Early in the work with onion yellow dwarf it was found that many plants which became inoculated during the current growth period failed to show visible infection until the bulbs

were regrown the following season. Such a condition may be the result of a number of influences the more important of which are perhaps the stage of growth of the plant and the growing condition of the plant at the time infection occurs. Plants which are infected late in the growing season or when fairly mature quite commonly fail to show disease symptoms during the current season a condition which is also true with stunted plants or plants in a poor state of growth.

A series of experiments were conducted to determine if such plants might serve as a source of inoculum for aphid vectors. On seven plants, in a poor state of growth and otherwise stunted at the time of exposure to infective aphids, which had failed to develop visible yellow dwarf symptoms, colonies of A. rumicis were confined for 24 hour periods and then transferred to young healthy onion plants. The results of these tests are shown in Table VII. It will be noted that of 34 healthy plants exposed only 7 developed visible symptoms and that these infections were obtained from 2 of the original 7 plants that were being used as a source of inoculum. These experiments, although of a limited scope, are sufficient to establish the fact that masking of symptoms does occur and that aphids feeding upon such plants may become infectious and spread the disease to healthy plants.

TABLE VII MASKED SYMPTOMS

No. of plants used in test.	No. healthy plts. exposed.	No. plts. infected.
1	5	0
1	3	0
1	4	3
1	7	0
1	4	0
1	5	0
1	6	4
Total 7	34	7

FIELD STUDIES ON APHID POPULATIONS

During 1932 a field station was maintained in the onion growing district at Pleasant Valley, Iowa and daily observations made in representative fields throughout the growing season for the purpose of determining the trend of aphid populations. Also, similar field observations were made at various times during the years 1930 and 1931 and from 1933 to 1935 inclusive. A more or less detailed account of these studies will be presented in the following discussion.

The first aphids to appear in the onion fields in noticeable numbers were pea aphids (M. pisi). They were first noted feeding on onion plants on May 10. On May 13, in fields located near alfalfa, an estimate based on counts made at various points placed the number of M. pisi at about 2500 per acre. The fact that this species of aphid spends its entire life on alfalfa and related plants, thus eliminating the necessity of a spring migration from a primary to a secondary host and the resultant rebuilding of a population, appears to be particularly conducive to the production of a heavy infestation in the early spring. Alfalfa fields in the area under observation became extremely heavily populated with the pea aphid during the first half of the month of May resulting in a general tendency of both alate and apterous

forms to fly and crawl about promiscuously in search of new less densely populated food plants.

In the majority of fields at Pleasant Valley several species of weeds, including lamb's quarter, clover, pursley, several grasses, along with a number of other plants, succeed in establishing themselves in the early part of the season, especially during abnormally wet seasons. As our records show, these plants become densely populated with various species of aphids, irrespective of size or location weeds in the field. In many cases 50 to 75 M. pisi were found on a small clover plant growing in the center of 20 to 30 acre onion fields. As many as 300 specimens of the bean aphid have been collected on a small shepherd's purse plant near the center of a large onion field.

Eventually, at least in most cases, these weeds are cut or pulled by the growers and the plant lice including nymphs, alate and apterous forms are left in the field with no source of food except the onion plant. In case the weeds are not destroyed early, they are soon overpopulated and become a center of migration in all directions.

Plant lice apparently have an inherent tendency to at least make an effort to feed on any plant upon which they happen to crawl or light and their chief criterion for determining the desirability or undesirability of a plant as a source of food appears to be by sampling it. In other words

the fact that an aphid finds one onion plant an unsatisfactory source of nourishment does not impress it with the fact that the next onion plant will be of the same nature. As a result, one plant louse in migrating through an onion field may insert its beak and feed to some extent on a number of plants. In one particular instance an alate M. pisi was observed to feed on 4 onion plants in succession in a period of 30 minutes and at the end of this time was crawling toward another. It was not uncommon to find from a few to 30 or more aphids feeding on a single onion plant near a recently cut weed.

In a field belonging to a grower who had made no effort to control yellow dwarf it was noted that diseased volunteer onions were growing in considerable numbers, as were also a variety of weeds, such as shepherd's purse, seedling box elder plants, and others. Many of these were heavily infested with aphids. During the first week of June (1932) the weeds were cut by the grower and an examination of the fields a few days later showed that large numbers of aphids were feeding on onions. In order to secure conclusive evidence that aphids become infectious by feeding on diseased plants under field conditions, 115 A. rumicis, found on diseased volunteer onions near recently cut shepherd's purse plants, were collected and confined on 6 disease free onion plants. At the end of two weeks 5 of these had developed typical yellow dwarf symptoms.

Following the time at which the weeds were cut frequent field observations showed that many new cases of yellow dwarf had developed with the diseased volunteer onions acting as centers of infection.

Another factor which has been observed to influence the rate of field spread of yellow dwarf has been the proximity to other vegetation such as alfalfa fields and certain vegetable crops which, as a rule, support a heavy aphid population during the spring months. Although no definite experiments have been made on this point, it has been noticed repeatedly that the incidence of yellow dwarf is unquestionably greater in onion fields adjacent to alfalfa or clover fields than in areas located at some distance from these legumes.

The Pleasant Valley onion growing district is located along the Mississippi River and comprises a narrow valley approximately 1/2 mile in width and 4 or 5 miles in length. It is bordered on the east by the Mississippi River and on the west by a series of hills or bluffs which act as somewhat of a barrier between the valley and the higher grain growing region to the west.

In this district there is, in addition to onions, a considerable acreage of other vegetables such as cabbage, melons and others which are favourable hosts for aphids. Also, there are rather extensive areas of waste land, partly

in the form of ditch banks and low lands near the river, which are unsuitable for cultivation. In such places grow a wide variety of weeds and trees many of which at times become heavily infested with aphids. It is quite evident, therefore, that in this area ecological factors are of such a nature as to encourage the development of heavy aphid populations of various species which, in the course of their wanderings and migrations, may serve as disseminating agents for the yellow dwarf virus.

Approximately 10 miles from Pleasant Valley is a district where from 150 to 200 acres of set onions are produced each year. Although yellow dwarf is known to have been present in this territory since 1928, no special efforts have been made to control the disease and it has not at any time attained such proportions as to cause serious commercial losses. Furthermore, during the last few years, there has been a gradual decrease in the per cent of infection and at the present time (1936) only a trace of the disease can be found. Why such a condition should exist in this region is not definitely known but it is quite apparently related to a scarcity of the insects which disseminate the disease under natural conditions.

In contrast to the Pleasant Valley district this district is located in the grain growing region where practically all of the land is either planted in small grain and corn or used as pasture land. Consequently, the plant complex appears to

be less conducive to the production of heavy aphid populations of various species during the onion growing season.

At Pleasant Valley, Iowa commercial onions are grown from both sets and seed, the latter being originally free from disease since evidence indicates that there is no seed transmission. However, the more susceptible growing period of seedling onions coincides with the maximum aphid activity resulting in the most favorable conditions for transmission of the virus from set onions, which have carried the disease over from the previous season, to seedling onions grown both for commercial purposes and for sets the coming year. Thus cultural practices in combination with the natural characteristics of plant lice are conducive to a perpetuation of the disease from year to year under uncontrolled conditions.

As has been observed by the writer as well as numerous other workers, the density of aphid population varies throughout the growing season. During the 1933 growing season, at Pleasant Valley, there was in general a gradual increase in plant lice up until the latter half of June. In August and September, due to parasites and predators along with other factors of the environment, the aphid population was in most cases reduced to inappreciable numbers, a notable exception being the melon aphid, (A. gossypii), which remained fairly numerous on cucurbits during these months. The most conspicuous

and generally extensive migration of any one species was that of H. atriplicis which began on the 11th of June and covered a period of about 10 days. On June 11 they were present in onion fields, as determined by making counts at various points, at the rate of 10,850 per acre. On June 12 individuals of this species were found to be present in some onion fields at the rate of 52,000 per acre. Since practically all of these seemed to be feeding at the time counts were made, an effort was made to determine whether or not there was a gradual shifting of the aphids throughout the fields. To secure this information all the aphids, so far as possible, were removed from an area of 315 square feet. Two hours later counts were again made on the same area and aphids were found to be present at the rate of 20,000 per acre. On June 13 the number of individuals had increased to 87,120 per acre. Set onions are planted at the rate of approximately 225,000 per acre which means that there were about 2-1/2 onion plants to every individual of this species found in the field. At first thought this might lead to doubtful conclusions in regard to the possibility of plant lice playing the role of natural vectors. However, the constantly shifting characteristics of aphids in an onion field which vary in rapidity among different species undoubtedly brings about a very thorough and intricate system of cross inoculation. This condition, combined with the ability of the aphids to obtain the infective

principle at one feeding and immediately transfer it to healthy plants, unquestionably appears to be particularly favorable for a very rapid spread of the virus.

Following the July and August scarcity of plant lice there was a gradual increase in abundance up until freezing temperatures occurred. Coincident with this increase, especially in the latter part of September and early part of October in Iowa, occurs the fall migration back to the primary or winter host. (An exception to this being M. pisi which has no alternate host). The apple grain aphid, R. prunifoliae, was especially abundant during late summer and fall in 1932. On September 19 in onion fields which had been planted in barley, after the onion crop was harvested, this species became very numerous, from a dozen to 50 or more being found on practically every barley plant examined. Many of these were winged forms. At the same time volunteer onions, regardless of whether they were growing nearby or 1/2 mile or more away from the barley fields, were usually harboring one or more winged forms of this species. In many cases as many as a half dozen were observed on one plant.

The significance of the heavy late summer and early fall aphid population in connection with yellow dwarf virus dissemination depends entirely upon the importance of volunteer onions in carrying the disease from one season to the next.

Evidence that volunteer onion may serve as an overwintering source for the virus will be presented in a succeeding

section of this paper (p.74).

Further information concerning aphid populations in onion fields was obtained by the use of "screens" consisting of frames, 2 square feet in area, covered over with ordinary 16 mesh screen door wire. Over each side of the screen was spread a thin layer of "tree tanglefoot" which served as a trap for any insects, especially smaller ones, which happened to come in contact with it. It was found necessary to replace the "tanglefoot" at about 2 week intervals because of the accumulation of various objects, including wind blown materials such as dust and small particles of trash, and miscellaneous insects of various species. These "screens" were placed near the center of large fields, in two different localities designated as locality A and locality B. At locality A the entire "screen" was composed of 4 units (each unit 2' x 2' in area), 2 facing north and south, one of which extended from 4 feet above ground surface upward and the other directly above it, and 2 facing east and west with the same arrangement as in the former. At locality B the "screen" was composed of 2 units placed in a manner similar to that in A and extending in height from 6 to 8 feet above the ground level.

Daily examinations of these "screens" were made throughout the growing season of 1932 and records made of the number of aphids trapped and wind direction, with a notation as to

whether there was a low, medium, or strong wind or no wind, as the case might be. The purpose of the latter was to determine in a general way the relative effect of air currents on aphid migration.

Aphids which have been trapped by means of "tree tanglefoot" are usually in a very much distorted and mutilated condition upon removal from the material, thus making definite identification very difficult in many cases. It was comparatively easy, however, to identify the more common and more abundant species. Perhaps the species most commonly taken were the pea aphid, M. pisi, the bean aphid, A. rumicis, the melon aphid, A. gossypii, and M. asclepiadis, a species occurring on milkweed. These species were taken more or less regularly during the spring months. A large number of other species were collected, some of which occurred in considerable abundance on different occasions. Among these may be mentioned the apple grain aphid, R. prunifoliae, the lamb's quarter aphid, H. atriplicis, the cabbage aphid, B. brassicae, the corn leaf aphid, A. maidis and several others.

That the abundance and duration of migration of winged forms is materially influenced by air current is indicated by the following records made, in connection with the above mentioned "screens", on June 25 and 26. "On June 25 no noticeable wind, 6 aphids collected from "screen" B, distributed as follows: S-4, N-1, W-1, E-0. June 26 fairly strong

wind from west, 155 aphids collected from "screen" B, distributed as follows: S-14, N-20, W-121, E-0". These results were duplicated in their essentials throughout the course of the experiment.

On 8 square feet of screen area located near the center of an onion field comprising approximately 100 acres, broken only by small drainage ditches and field roads, 1697 plant lice, including various species, were collected during the month of June 1932. Based on these figures, which do not take into account the individuals which migrate at heights of less than 4 feet and more than 8 feet, several hundred thousand winged aphids crossed each acre of onion field in the locality under observation during the month of June. It is a fairly well established fact that aphids, whether they be spring, summer, or fall migrants, when influenced by the migratory impulse in combination with the effects of air currents and other environmental factors, exercise little if any selectivity either with respect to direction of movement or duration of flight. Chance therefore seems to be the chief controlling factor operating toward the location of new host plants by the migrating insects.

Four "tanglefoot screens", similar to the ones described above and comprising a total of 10 square feet of surface, were set up in the experimental plots at Ames during April 1933. From these "screens", between April 2 and April 22

inclusive, a total of 2336 aphids of various species were taken. It is of interest to note that there were included among these considerable numbers of individuals of non-migratory species such as M. alhambri and M. asclepiadis.

These data convincingly indicate that although a species of aphid may be non-migratory, i.e. normally confined to one species or group of closely related species of plants throughout the year for feeding purposes, many winged individuals migrate presumably in search of more favourable food plants of the same species. During their migrations and fortuitous wanderings these individuals either by chance or necessity may rest upon and imbibe the juices of plants which can not serve as a permanent host.

The records given and the points covered in the preceding pages concerning aphid populations in onion fields seem to justify the contention that plant lice occur in onion fields in sufficient abundance and at the proper time to serve as important vectors in the natural dissemination of onion yellow dwarf.

SOME PROPERTIES OF THE VIRUS

Longevity of Virus in Dried Infected Onion Leaves

Yellow dwarf infected onion leaves were collected and stored at 30° C. and at 5° C. At varying intervals plant lice were confined on these leaves and then transferred to healthy onion plants. In order to induce the insects to feed on such material it was found necessary to place the leaves in high humidity chambers until they became moistened. The results of these test are shown in Table VIII. By reference to the table it will be noted that at a temperature of 30° C., of 6 plants exposed to aphids which had fed on dried leaves for a period of 24 hours, 4 or 66.6 per cent became diseased and at the end of 56 hours 7 out of 8 healthy plants exposed became diseased. Of 3 plants exposed after storage of leaves for 196 hours, 1 developed mosaic symptoms and none thereafter. Of the leaves stored at 5° C. one successful inoculation was obtained after a 214 hour period. As was expected, considerable difficulty was experienced in inducing aphids to feed on dried onion leaves but this problem was overcome to some extent by using large numbers of aphids.

TABLE VIII LONGEVITY OF VIRUS IN DETACHED ONION LEAVES

No. hrs. leaves stored before using	No. plants exposed	No. plants infected	Storage Temp.
24	6	4	30°C.
56	8	7	"
96	1	0	"
120	4	0	"
144	3	1	"
196	3	1	"
216	10	0	"
168	2	0	5°C.
192	2	0	"
214	2	1	"

Systemic Distribution of Virus

In general, virus agents associated with plant diseases have been found to occur in all parts of the infected plant. Experiments were conducted for the purpose of determining if the virus could be shown to be present in all parts of the onion plant by using aphids as the transmitting agent. The results of the test appear in Table IX.

"Presence of virus in dormant diseased bulbs" - Aphids were confined on the fleshy portion of diseased bulbs for 24 hour periods and then transferred to healthy growing onion plants. Of 32 healthy plants inoculated in this manner none became diseased. In another experiment the fleshy portion of diseased bulbs was removed and the growing point used as a source of inoculum. Of 48 healthy growing plants exposed, 8 developed yellow dwarf symptoms. The results of these tests which demonstrate the presence of the virus in the growing point of diseased dormant bulbs in virulent form but fail to show a similar condition in the fleshy portion of such bulbs, appear in Table IX., A.

"Infection of dormant bulbs by exposure to viruliferous aphids" - Plant lice were confined on diseased growing onion for a period of 24 hours and then transferred to the growing point of healthy dormant bulbs (fleshy scales partly removed) for a similar period. The bulbs were then planted and observed

for the development of yellow dwarf symptoms. Of 44 healthy bulbs exposed to infection in this manner 12 produced diseased plants. The results of these tests appear in Table IX, B. Attempts were made to inoculate dormant bulbs by confining viruliferous aphids on the fleshy portion but no successful transfers were obtained.

"Presence of virus in mature leaves which show no visible symptoms of yellow dwarf". - The leaves of plants which are fairly mature at the time infection occurs do not as a rule develop yellow dwarf symptoms. In order to determine whether or not the virus is present in an active form under such conditions, aphids were confined on non-visibly infected leaves of a plant, showing in the younger growth typical disease symptoms, and then transferred to healthy growing plants. Of 19 healthy plants exposed, 10 became diseased. (See Table IX, C.)

"Infection of healthy plants by inoculating mature leaves".- Colonies of aphids which had fed on diseased plants for a 24 hour period were confined on mature or old leaves of healthy plants. Of 26 healthy plants exposed 9 became diseased. The old leaves of these plants upon which the aphids were confined, however, remained healthy in appearance (See Table IX, D.)

"Presence of virus in stem, bulb, and roots of growing diseased plants". - Aphids were confined on the stem, bulb,

and roots of diseased plants and then transferred to young healthy onion plants. (See Table IX, E.) It will be noted that of 9 plants exposed to aphids which had fed on the stem of infected plants 4 developed yellow dwarf symptoms. From the aphids which fed on bulbs and roots, 9 and 8 healthy plants, respectively, were exposed and one of each became diseased. As would be expected considerable difficulty was experienced in inducing aphids to feed on the bulb and roots, which probably accounts for the low percentage of takes.

TABLE IX SYSTEMIC DISTRIBUTION OF VIRUS IN GROWING PLANT AND DORMANT BULB

Source of inoculum	No. healthy plts. exposed	No. infected
A. Presence of virus in various part of dormant diseased bulbs.		
Fleshy layer next to growing point	32	0
Growing point	49	8
B. Infection of dormant bulbs by exposure to viruliferous aphids.		
Growing diseased onion.	44	12
C. Presence of virus in mature leaves which show no visible symptoms of disease.		
Mature leaves showing no disease symptoms	19	10
D. Infection of healthy plants through mature leaves.		
Growing disease onion	26	9
E. Presence of virus in stem bulb and roots of growing diseased plants.		
Stem	9	4
Bulb	9	1
Roots	8	1

OVERWINTERING OF YELLOW DWARF VIRUS AND ITS RELATION TO INSECTS.

All evidence to date indicates that host plants of the onion yellow dwarf virus, other than the cultivated onion, do not occur in Iowa. Evidence which indicates that it is not a seed borne or soil borne disease has also been presented by Henderson (1935). That insects might offer an overwintering source for the virus is realized but supporting evidence has not been established. In view of this situation the "carry over" of the virus from year to year depends entirely on onion bulbs either in storage or as refuse in the field.

In Bulbs

Each summer during the period of 1928 to 1933 inclusive several hundred bulbs from yellow dwarf diseased plants have been collected at Pleasant Valley and brought to Ames for use during the following winter and spring as a source of inoculum in insect transmission tests. From these infected bulbs, which were planted at various times, plants showing unmistakable yellow dwarf symptoms were grown. That onion bulbs including sets, mother bulbs and cull onions in the field

are an important overwintering source for the yellow dwarf virus has been repeatedly demonstrated and further comment on this problem is not considered necessary at the present time.

In Volunteer Onions

During the fall of 1933, 58 volunteer onion plants in the experimental plots at Ames, were exposed to viruliferous aphids which resulted in 40 successful inoculations, as evidenced by the development of typical disease symptoms. These plants continued growth as long as weather conditions were favorable and throughout the winter remained undisturbed and exposed to normal out-door conditions. The following spring (1934) these plants resumed growth and exhibited typical yellow dwarf symptoms. From aphids confined on these plants 20 healthy plants were inoculated resulting in 13 successful transmissions.

On numerous occasions diseased volunteer onions found growing in the field at Pleasant Valley and at Ames have been used as a source of inoculum for infecting healthy plants with successful results. It is, therefore, a well established fact that the virus can overwinter in Iowa in onion bulbs under field conditions.

The "carry over" of the yellow dwarf virus in volunteer

onions from year to year is of considerable importance in perpetuating the disease. As a general rule a heavy growth of volunteer onions is produced in the infected area each fall (Sept. and Oct.) and this coincides to some extent with the fall migration of aphids. For the last several years fall volunteer onions have been examined for the presence of plant lice and on some occasions from 1 to 15 plant lice were found on practically every plant. These individual aphids, as determined by careful observation, were feeding on the onion plants for considerable periods of time. In many cases it was conspiciously noticeable that the percentage of visible yellow dwarf infection was considerably higher than in the commercial crop. This apparent increase may have been influenced by the 3 following factors: (1) masking of symptoms in plants which became infected late in the growing season, and the development of symptoms in these plants during the second growth period, (2) a tendency of yellow dwarf infected bulbs to be less susceptible to a long dormant period, and (3) the heavy population of plant lice which is found in onion fields during the fall migratory period. The following remarks are taken directly from field notes which were made on October 7, 1933 in connection with a field which had been planted with home grown sets but showed less than 1 per cent of visibly infected plants: "(1) On 27 square feet, 100 volunteer onions and 25 of these diseased, (2) On

6 square feet, 25 volunteer onions - 13 of these diseased, (3) On 6 square feet, 36 volunteer onions and 6 of these diseased. In one case 14 winged aphids(R. prunifoliae) taken from a single volunteer onion. Hardly a plant could be found which did not harbor one or more winged aphids".

It should be noted that the above mentioned samples were taken at random in a fairly large onion field (approximately 20 acres). All onion plants in a given area were counted and the number showing disease symptoms recorded. These data indicate that conditions favorable for a rapid spread of infection are present among volunteer onions and that these plants are able to survive the winter and develop into a further source of infection the following spring.

It has been observed that many of the volunteer onions, at least in fields belonging to certain growers, which resume growth in the spring are left in the field throughout the growing season. Such cultural practices are favorable for perpetuating a disease which otherwise offers some promise of complete eradication.

INTERTRANSMISSIBILITY OF THE VIRUS

Inoculation of Plants Other Than the Cultivated Onion.

In order to understand the problems related to natural dissemination of a virus disease it is of considerable value to establish its host range. An experiment was therefore conducted in which a large number of both wild and cultivated plants were exposed to viruliferous aphids.

Colonies of aphids were confined on diseased onion for a period of 24 hours and then transferred to the experimental plants (not onion) for 24 to 48 hours, and in some cases for a considerably longer period. The result of these tests appeared in Table X. It will be noted that of a large number of plants exposed none developed what was considered as yellow dwarf symptoms.

In a number of cases certain of these plants developed a mosaic like appearance somewhat suggestive of a virus disease. With all plants listed in Table X a number of attempts, especially with those plants which seemed to exhibit a mosaic appearance, were made to infect healthy onion plants by caging aphids on these plants for a period of time and then transferring them to healthy onions. In no cases, however,

was transmissibility demonstrated. It is possible that the chlorotic condition in these plants was caused by some abnormal physiological condition of the plant or possibly to some virus agent other than the yellow dwarf virus.

In addition to the plants mentioned in Table X, attempts were made to inoculate a number of other both cultivated and wild plants. Among these were croecus, (Crocus biflorus, Mill.), plaintain (Plantago ssp.), corn, (Zea mays L.), lamb's quarter (Chenopodium album L.), dock, (Rumex ssp.)alfalfa (Medicago sativa L.), milkweed (Asclepias ssp.), and various species of grasses. Since no evidence was obtained to indicate that any of these plants are susceptible to yellow dwarf, the details of these experiments will not be given.

TABLE X. INOCULATION OF PLANTS OTHER THAN THE CULTIVATED ONION BY MEANS OF APHIDS.

Common Name	Scientific Name	Family	No. Plts. Exposed	No. Infected
Wild garlic	Allium canadense L.	Liliaceae	64	0
Shallots	A. " ascolonicum L.	"	37	
Leek	A. " porrum L.	"	16	
Galic	A. sativum L.	"	10	
Chive	A. schoenoprasum L.	"	24	
Field garlic	A. vineale L.	"	32	
	Lilium auratum Lindl.	"	6	
	L. canadense L.	"	2	
	L. elegans Thunb.	"	2	
	L. Henryii Baker	"	6	
	L. japonicum Thunb.	"	4	
Easter lily	L. longiflorum Thunb.	"	11	
	L. regale Wils.	"	4	
	L. rubrum L.	"	16	
	L. speciosum L.	"	15	
	L. superbum L.	"	5	
	L. tenuifolium Fisch.	"	12	
Tiger lily	L. tigrinum Ker.	"	8	
	L. umbellatum Pursh.	"	5	
Tulip	Tulipa gesneriana L.	"	8	
Jonquil	Narcissus jonquilla L.	Amaryllidaceae	26	
	N. odorus L.	"	26	
Daffodil	N. pseudo-narcissus L.	"	24	
Chinese sacred lily	N. tazetta L.	"	17	
Iris	Iris sp.	Iridaceae	18	
Gladiolus	Gladiolus sp.	"	12	

Healthy onion plants were exposed to the feeding of aphids which had been previously confined on plants (not onion) either known to be infected with a virus disease or exhibiting virus disease like symptoms. Among these were: Easter Lily.(L. longiflorum), milkweed (Asclepias sp.), tomato (Lycopersicum esculentum Mill.), Tulip (Tulipa sp.) cucumber (Cucumis sativus L.), alfalfa (Medicago sativa L.), aster (Calistephus chinensis Nees.), sweet clover (Melilotus alba Desr.), wild lettuce (Lactuca canadensis L.), plantain (Plantago sap.), dock (Rumex sap.), purslane (Portulaca oleracea L.), corn (Z. mays), garden pea (Pisum sativum L.), iris (Iris sp.), garlic (A. sativum), leek (A. porrum), and a number of others. No definite indications of intertransmissibility were obtained.

In the Pleasant Valley onion growing district are a number of localities in which wild garlic, commonly referred to as wild onion, (Allium canadense L.) grows quite profusely from year to year. It seemed quite logical to suspect that this closely related species of the cultivated onion might serve as a reservoir of the yellow dwarf virus. A large number of experiments were therefore conducted to test this hypothesis through the agency of aphid vectors, but in no case was a relationship established. In some instances, wild garlic plants, which were inoculated by means of aphids that had

previously fed on yellow dwarf infected onion plants, developed a mild chlorotic condition somewhat suggestive of a virus disease. Attempts to infect healthy cultivated onion plants by using such plants as a source of inoculum, however, were unsuccessful. Likewise, 32 plants of field garlic (Allium vineale L.) inoculated by means of infective aphids gave negative results.

During the spring of 1932 daily observations were made by the writer in and around onion fields in the infected area for the purpose of locating any wild or cultivated plants which showed mosaic-like symptoms suggestive of yellow dwarf. A number of plants displaying such symptoms were discovered and in practically all instances attempts were made to inoculate healthy onion plants by means of plant lice which had been previously confined on the suspicious plants. No successful transfers were obtained.

Varietal Intertransmissibility

In addition to the varieties of onions which are most commonly grown on a commercial scale in the yellow dwarf infected area, consisting mainly of the Red Globe and Yellow Bottleneck strains, yellow dwarf has been experimentally transmitted, by means of plant lice, in the greenhouse and experimental plots at Ames, to the following varieties of

cultivated onion: River Side Sweet Spanish, Yellow Flat Danvers, Prizetaker, Shallots or Yellow Multipliers, Red Bottom, Yellow Bottom, Yellow Potato Onion, White Multiplier, White Bottom, Brown Australian Flat, Extra Early Flat Red, Large Red Weathersfield, White Portugal or Silverskin, Japanese or Ebenezer, White Queen, Strassburg or Yellow Dutch, Fancy Yellow Globe Danvers, Giant Gibraltar, Ailsa Craig, Crystal White Wax, White Barletta, Vaughn's Pickling, Mammoth Silverking, Yellow Globe, Southport White Globe, Yellow Bermuda, Extra Early White Queen, American Flag, Yellow Valencia and White Valencia.

The number of healthy plants exposed in each of the above mentioned varieties ranged from 10 to 50 and the per cent of visible infection from 30 to 90. All varieties tested were found to be susceptible. In most cases no attempt was made to determine the degree of varietal susceptibility or tolerance to the disease. As previously mentioned it was noted, however, that the variety Riverside Sweet Spanish seemed to possess a certain degree of resistance.

INTRACELLULAR ABNORMALITIES ASSOCIATED WITH YELLOW DWARF OF ONIONS

In considering the possibilities of this problem, it was hoped that a fairly complete cytological study of the virus-affected tissue could be conducted, but insufficient time made it necessary to confine efforts to a more limited field, that is, to a determination of the presence of intracellular bodies.

The presence of cell inclusions in plant tissue was first reported by Iwanowski in 1903 in connection with a study of tobacco mosaic. Since that time many workers have made contributions to this phase of the plant virus problem, the reports of which are accompanied in some cases (Cook 1925, Goldstein 1926, Smith 1934) by elaborate literature reviews. As a result it seems desirable at this time to review briefly only a few of the publications which are closely related to the present problem and pertain particularly to monocotyledonous plants.

In 1910 Lyon described intracellular bodies in sugar cane tissues affected with Fiji disease. These structures were later described in detail by Kunkel (1924). Kunkel (1921) in discussing the causative agent of corn mosaic described irregularly shaped bodies which were closely associated

with the host cell nucleus and were believed to be living organisms. About a year later Kunkel (1922) reported somewhat similar bodies in mosaic diseased tissue of Hippeastrum equestre Herb. and of sugar cane. Mc Kinney, Eckerson and Webb (1923 and 1924) reported that irregularly shaped intracellular bodies were associated with mosaic in Hippeastrum Johnsonii Bury and with rosette and mosaic in wheat. Cook (1925) found intracellular bodies in sugar cane affected with mosaic but they were by no means abundant or conspicious and occasionally they were entirely absent in severely diseased tissue. In some cells in which there were peculiar bodies no nuclei were recognizable. In some cells of both diseased and apparently healthy plants he found two or more nuclei present. Eckerson (1926) reported the presence of intracellular bodies resembling flagellates in mosaic infected wheat and H. Johnsonii. Sheffield (1934) has succeeded in inducing the early symptoms of mosaic by treating healthy tissues with certain protoplasmic coagulating substances. By treatment of healthy tissues with molybdic acid he induced the formation of cytoplasmic bodies similar to the intracellular bodies in tissues affected by Aucuba mosaic.

Materials and Methods

The investigations were confined to the intracellular

bodies associated with the yellow dwarf disease of the onion and involved a comparison of the protoplasts of diseased and healthy plants. The material for examination was selected from plants grown in both field and greenhouse, some of which had been experimentally inoculated by means of aphids while others had become infected under natural conditions in the field. Some of the diseased plants had carried the infection through 3 seasons of growth, whereas others had been infected recently. Also, material was included from plants in different stages of development.

Studies were made of both fresh and fixed material. The living material was sectioned free-hand. Some of the sections of living materials were mounted in water and examined without further preparation, whereas other sections were killed, stained, and mounted in glycerol.

The killing solutions tried were chromacetic, Bouin's and the alcohol-formalin-acetic acid solution. These solutions were used in combination with a number of stains - namely, hematoxylin, safranin gentain violet, analine blue, and haemalum. The chromacetic fixing solution (one part of one per cent chromic acid plus one part of one per cent acetic acid) and haemalum made the best combination. Both paraffin and free-hand sections fixed and stained as described above were employed, but free-hand sections were more satisfactory.

The observations and also the photomicrographs of the intracellular bodies were made with a spencer microscope equipped with oil immersion and 4mm. objectives and 12X ocular.

Description of Intracellular Bodies.

As a general rule the intracellular bodies were rather sparse even in the onions exhibiting extremely severe macroscopic symptoms. Only occasionally were more than 3 or 4 bodies present in the field of the oil immersion objective. They were also very irregularly distributed, being entirely absent in some areas and present in considerable numbers in other areas of the same section. The number of intracellular bodies present in a cell was usually not more than one or two (Plate II, figs. 6, 8, 9), but occasionally 4 or 5, in which case they were commonly in a close group (Plate II, fig. 5). Apparently the intracellular bodies in onions infected with the yellow dwarf virus are not so numerous as has been reported in sugar cane, corn, and some other plants having a mosaic disease.

The intracellular inclusions varied much in size, shape, structural appearance, and in their position with reference to the nucleus and to each other. They were usually similar to the nuclei in size and shape (Plate II, figs. 6, 8). Often they could not be distinguished from nuclei with certainty (Plate II, fig. 4). Some were much larger than the nucleus

(Plate II, figs. 2, 3). Usually they were more vacuolate and took less of the stain than the nuclei (Plate II, figs. 3, 10), but often in structural features and their behavior with reference to stains they were indistinguishable from nuclei (Plate II, figs. 6, 8). They were nearly always either close to or in contact with the nucleus, either directly (Plate II, figs. 3, 6, 9, 10) or by connections of similar material (Plate II, figs. 4, 8). Sometimes they partially surrounded the nucleus (Plate II, fig. 2). Where several were present in the same cell they were usually in a close group which was either close to or in contact with the nucleus (Plate II, figs. 5, 7, 11). The members of the group were usually in direct contact but occasionally they were joined by narrow connections (Plate II, fig.4).

In comparing material from apparently healthy and diseased plants the difference in respect to intracellular bodies was not so clear cut and striking as was anticipated, for cells were occasionally found in the apparently healthy tissues which were either multinucleate or possessed bodies similar to some of the extra bodies in the diseased tissue. They deviated from the usual condition of the healthy tissue shown in figure I. The cells with extra bodies were much less frequent and less variable in the healthy plants, but it was not impossible to find in the supposedly healthy tissues duplicates of a number of the types of intracellular bodies present in the diseased tissues.

DISCUSSION

The distribution of yellow dwarf in Iowa is confined to the Pleasant Valley onion growing district, a condition which appears to be related to cultural practices. In this district a considerable proportion of the commercial onion crop is ordinarily grown from sets, whereas, in other onion growing regions of the state, seed is the principal source of commercial plantings. Evidence to date indicates that the disease is not seed borne, thus the principal overwintering source of the virus--namely, bulbs, is eliminated in areas where the commercial crop is produced directly from seed.

Some virus diseases of plants are known to attack only a small number of more or less closely related species while a few have been shown to have a rather wide host range, such as aster yellows and ring spot disease of tobacco. In contrast, information available at the present time indicates that the onion yellow dwarf virus is highly specific and under normal conditions is not known to attack any plant other than the cultivated onion (Allium cepa L.). That such a condition should actually exist, however, hardly seems tenable and it is believed that eventually additional host plants will be located either in this country or such other places where the disease may occur. The rather sudden occurrence of the malady in the Pleasant

Valley onion growing district indicates a relatively recent introduction from some outside source.

Although inoculable, the virus of onion yellow dwarf is not considered as being of a highly infectious nature and it can not be spread by ordinary contact. Man, insects, and other animals do not serve as passive conveyors of the disease nor can it be disseminated mechanically on insect cages and farm equipment.

All evidence to date indicates that aphids, which are only incidently associated with the onion plant, are responsible for the natural dissemination of the disease producing agent. In spite of the apparent inconsistency of this phenomena with the majority of other known insect-virus associations it is believed that sufficient experimental and observational data have been accumulated to demonstrate the feasibility of such a condition.

In a recent publication Baumeyer and Kearns (1936) report successful transmission of bean mosaic by 11 species of aphids and they concluded that others would quite likely prove to be vectors. According to these workers, aphids were not found to normally colonize on the bean plant and if confined on such plants from 48 to 72 hours they usually died. Attempts to transmit bean mosaic by means of other insects were unsuccessful.

Another somewhat similar example is that of sugar cane mosaic. Brandes (1920), Chardon and Veve (1923) and others have quite conclusively demonstrated that in Puerto Rico the corn leaf aphid, (A. maidis), which does not normally occur on sugar cane, plays an important role in the field dissemination of this disease.

A correct understanding of the importance of aphids in field dissemination of the onion yellow dwarf virus depends largely upon a knowledge of the life history and habits of aphids in general. The behavior and migration of these insects are governed largely by their host specificities, periodic or seasonal host restrictions, numerical abundance, capacity for reproduction, and availability of food plants. The fortunes of aphid colonies vary with weather conditions, with prevalence of aphidophagous fungi, and with the abundance of predacious and parasitic insect enemies and other factors of the environment. Some species are monophagous, whereas other forms are polyphagous and feed with various degrees of success upon many different kinds of plants.

With favorable food plants, optimun conditions of temperature, humidity, and other biotic conditions during the spring and summer, plant lice multiply very rapidly and often thickly populate and overrun their host. This over-crowding, together with the age and varying degrees of succulency of food plants, tends to accelerate migration. In all species,

however, and, especially in the winged individuals, there is an inherent urge to wander so that under field conditions migration occurs almost incessantly. Thus, both monophagous, and polyphagous species wander about in the spring, summer, and fall in search of new and less densely populated host plants during which they may feed to some extent upon any plant with which they come in contact. The onion seems to serve largely as a place to rest and a temporary source of nourishment during their fortuitous wanderings and migrations. Evidence indicates that such a relationship, however, is sufficient to affect a spread of the virus from diseased to healthy plants.

Plant lice acquire the causal agent during the first feeding upon diseased onion and immediately thereafter are capable of infecting healthy plants, thereby eliminating the hazards that necessarily would be encountered should a prolonged incubation period in the insect be obligatory. If there is a latent period, it is extremely short, and aphids seem to have very little, if any, more than an accidental connection with yellow dwarf virus.

The experiments presented in this paper have shown that inoculated aphids retain their infective power for only a relatively short time. Whether this condition is due to an attenuation or destruction of the virus within the insect or due to a passage through the body and final elimination is now known.

It is of interest to note that a species of aphid, Fullawayella formosana Takah., has been reported from Formosa (1921) as occurring in onions and some related plants. Essig (1935) recently described a species of aphid, Micromyzus alliumcepa Essig, from California which attacks the onion but in a subsequent report (1936) he states that M. alliumcepa is now considered as synonym of F. formosana. In California the onion aphid has been found to attack dormant bulbs as well as the growing plant and in addition is known to have caused severe injury to chive and leek. Further information concerning the geographical distribution of the onion aphid is not known to have been published but it appears quite likely that its present range will be extended and that it will be found to already occur in other regions. The importance that such an insect might have in the dissemination of onion yellow dwarf is quite apparent.

Since aphids do not normally occur on onion plants in Iowa, it may appear somewhat doubtful as to whether or not field dissemination of onion yellow dwarf could be attributed to or explainable on the basis of any one species or group of species of aphids. Field observations and experimental data are unquestionably sufficient to conclude, however, that practically any species of aphid is a potential vector of the disease. In Iowa there are probably more than 300 species of plant lice any one of which may be a contributing factor

toward the final results of field dissemination.

It is a well established fact that the virus of yellow dwarf overwinters in diseased sets, mother bulbs, and commercial onions. Such bulbs, together with diseased culls thrown in refuse piles and dump heaps or left in the field, may serve as sources of inoculum for the vectors the following season. The relation of such bulbs to the spread of yellow dwarf is quite evident. Since no host plant other than the cultivated onion has yet been found in Iowa, the diseased onion bulbs appear to be the only reservoir of the overwintering virus.

The control of onion yellow dwarf, in common with other diseases which depend on insects for natural dissemination, is either a matter of breaking the association of the vectors and the disease producing agent or development of resistant or immune varieties. Up to the present time the latter method does not seem to have offered any promise of immediate results. There appears to be little prospect of any practical form of general attack upon the aphid vectors being successful in controlling the disease. Thus, the only alternative is to remove or eliminate the source of inoculum which is being accomplished through the use of disease free seed stock grown in non-infected areas in combination with an application of ordinary field sanitation measures.

No definite conclusions were made concerning the intracellular bodies in the onion. At least two features suggested

that they were of nuclear origin - namely, their frequent contact with or close proxmity to the nuclei and the fact that they were often very similar to nuclei in form, structure, and in reaction to stains. In fact, sometimes they were indistinguishable from nuclei. Such bodies as shown in (Plate II, figures 6, 8, 9, and 10) may be interpreted either as nuclei in the process of amitotic division or as a nucleus and an intracellular body in close contact. The clusters of bodies in (Plate II, figures 5, 7, and 11) may be the result of repeated nucleur division and differences in the subsequent growth or degneration of the daughter nuclei.

It is possible that the onions selected as representatives of healthy plants were not entirely free from the yellow dwarf virus but did not manifest the ordinary recognizable symptoms. If these onions were healthy then the presence occasionally of cells which were evidently multinucleate would indicate a tendency of onion cells toward the multinucleate condition. The presence of a virus in the protoplast of the cell would probably enhance the tendency of the nucleus to divide and also abnormalities in the form, size, and structure of the nuclei.

There is also the possibility that the intracellular bodies are formed from the cytoplasm in response to the effect of the virus. Sheffield's (1934) work in which he induced the

formation of bodies in the cytoplasm of healthy tissues by the use of chemicals makes such an explanation tenable. That the intracellular bodies in the onion are results of the action of the virus on the protoplasm is more in accord with the nature of the bodies than the theory that they are organisms or substances that cause the disease.

SUMMARY AND CONCLUSIONS

Yellow dwarf is a virus disease of the mosaic type affecting the cultivated onion. In many cases, however, and especially in the advanced stages of the disease, chlorosis is more or less general and suggests the yellows type of disease.

In transmission experiments the virus was transmitted from diseased to healthy plants by more than 50 species of aphids. All transmission tests with other insects, with two exceptions, gave negative results.

The incubation period of the disease in the plant was found to be relatively short, ranging from 7 to 12 days in most cases with an average of 10.3 days.

It was determined that plant lice acquire the causal agent during the first feeding on diseased onion plants and immediately thereafter are capable of infecting healthy plants.

Experimental evidence convincingly demonstrates that if there is a period of delayed infectivity in the insect it is extremely short (only a few minutes) and that infective aphids, feeding upon either healthy susceptible hosts, immune hosts, or confined without food become non-viruliferous within the course of a few hours.

Although the onion plant serves as only an accidental

or transitory host of aphids in Iowa, field observations made in the infected area show that they are present in sufficient numbers and at the proper time to play an important role in field dissemination of the disease.

Since no host plants of onion yellow dwarf, other than the cultivated onion are known, and since indications are that it is neither seed nor soil borne, the only known overwintering source of the virus is the bulbs, both in storage and in the field. In view of this situation control may be accomplished by complete destruction of left-over bulbs in the field coupled with planting of disease free sets grown in non-infected areas.

Transmission experiments with a large number of species of plants other than the cultivated onion resulted in no successful transfers. The disease was transmitted to more than 35 varieties of cultivated onions by means of plant lice.

Under certain greenhouse and field conditions the symptoms of yellow dwarf may be completely masked and not visible until after the bulbs have undergone a rest period and have been regrown. Experiments were conducted in which it was found that such plants, can serve as a source of infection for plant lice, which, when so inoculated, can convey the disease to healthy onions.

Intracellular bodies were found in the tissues of onions affected with the yellow dwarf virus.

The intracellular bodies were not numerous and were very irregularly distributed.

They were usually either in contact or close to the nuclei.

Their position with reference to the nucleus of the cell and their frequent close similarity to nuclei suggested that they were of nuclear origin, possibly through amitotic nuclear division.

In tissues from apparently healthy plants, cells that were evidently multinucleated were found occasionally and some cells were observed in which bodies were present that differed somewhat from typical nuclei and that were similar to some of the types of intracellular bodies in the diseased onions.

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Plate I

Onion plant showing (a) typical feeding injury by the onion thrips and (b) yellow dwarf disease.

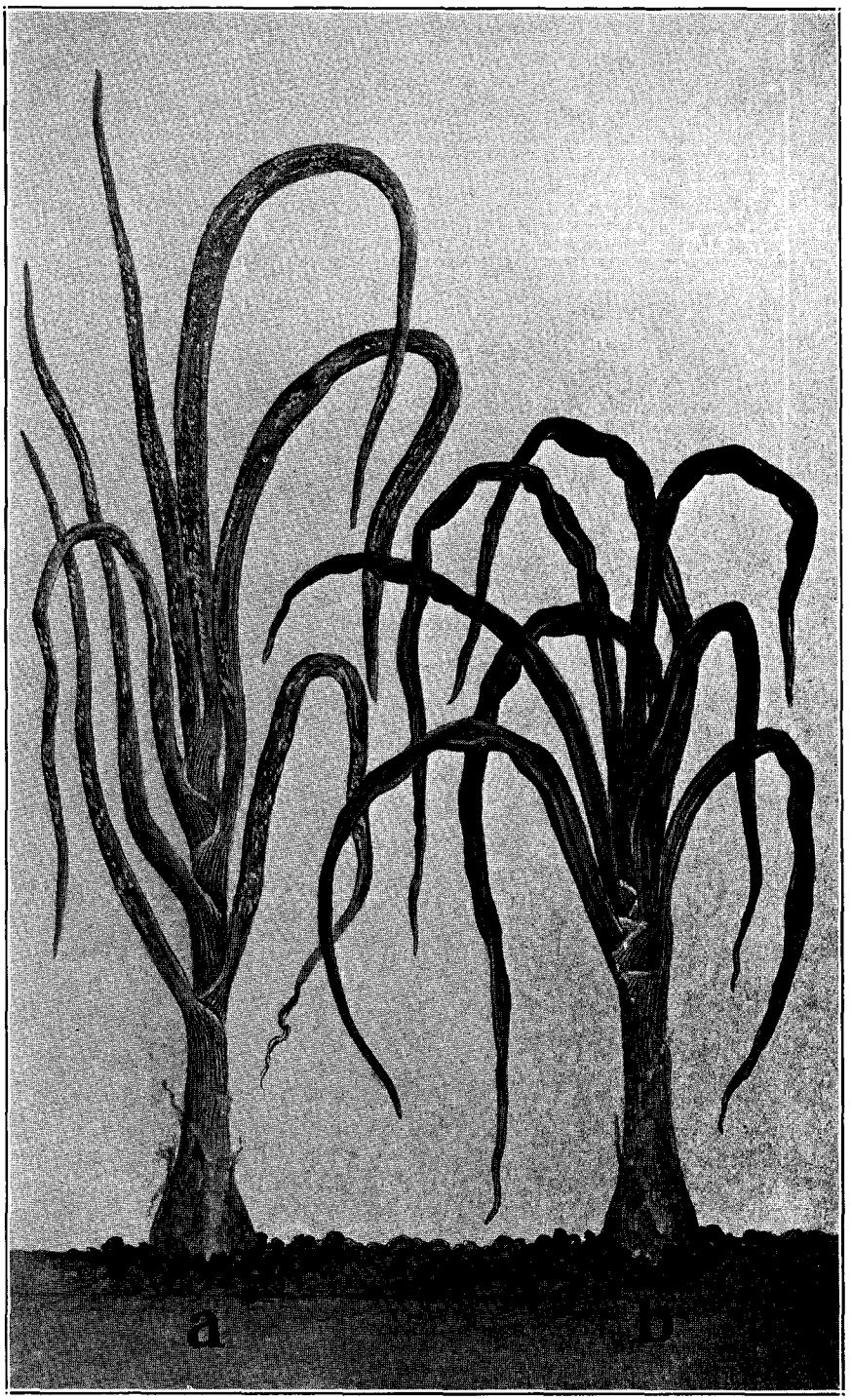

Plate I

Plate II

All magnifications X 300-400

Fig. 1. Cross section showing nuclei of healthy tissue.

Fig. 2. A large intracellular body partially surrounding the nucleus.

Fig. 3. A large foreign body closely applied to the host cell nucleus.

Fig. 4. Three closely associated intracellular bodies of which the darker one is probably the nucleus.

Fig. 5. A large, deeply staining amoeboid mass in which it is impossible to identify the nucleus.

Fig. 6. Section showing the relative distribution of the abnormal structures of chlorotic tissue.

Fig. 7. A large intracellular body completely separated from the host cell nucleus.

Fig. 8. An intracellular body and nucleus connected by protoplasmic strand.

Fig. 9. An intracellular body and nucleus joined by broad connection.

Fig. 10. An enlarged body less intensely stained than the nucleus.

Fig. 11. Three closely associated intracellular bodies of which the exact nature and relationship is not known.

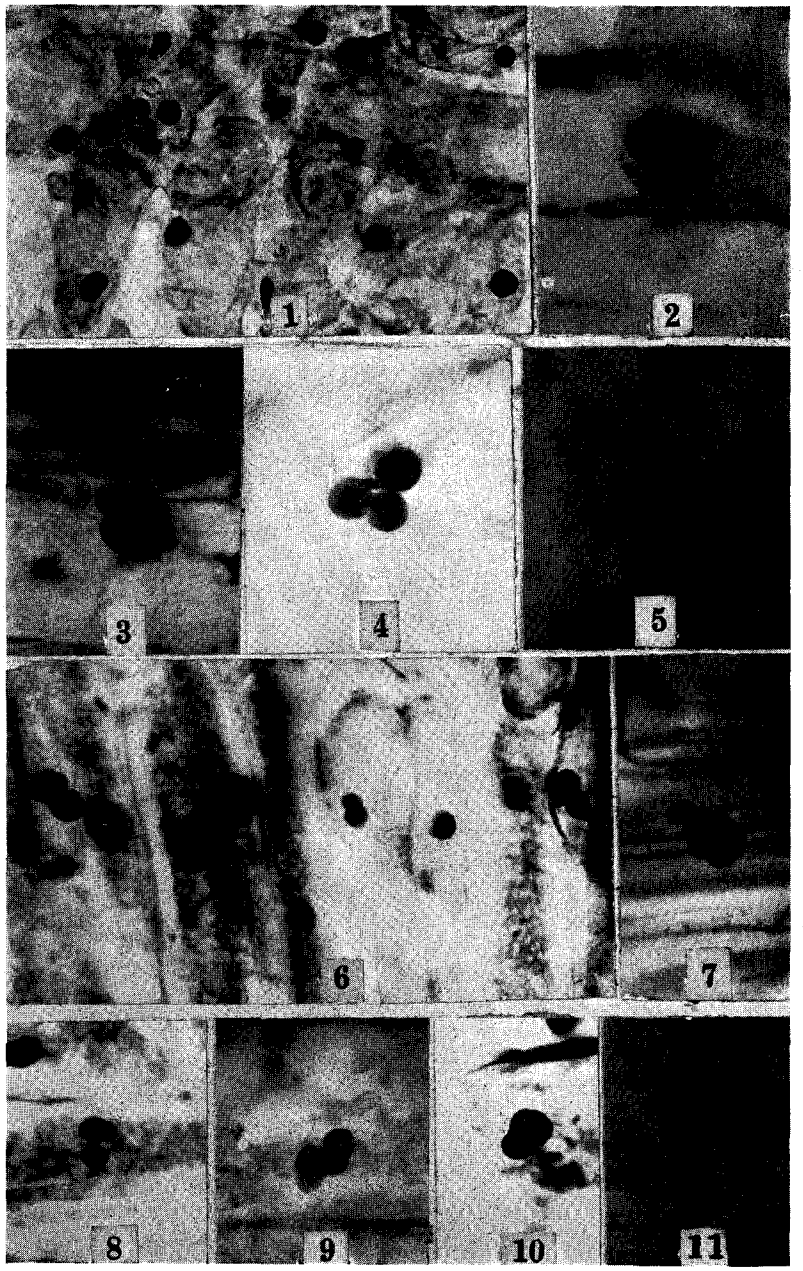

Plate II

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